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Original Paper

Enhanced Sensitivity of Small Cell Lung Cancer Cell Lines to Cisplatin and Etoposide After Infection with Adeno-associated Virus Type 2

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In previous studies we have reported the sensitisation of human tumour cells to gamma irradiation and chemotherapeutic drugs upon infection with the human non-pathogenic adeno-associated virus type 2 (AAV-2) *in vitro* and *in vivo*. Treatment of small cell lung cancer (SCLC) is consistently hampered by relapses due to the selection of chemotherapy-resistant cell clones. Hence, we were interested to test whether selection of chemotherapy-resistant SCLC cells might be reduced or even prevented if chemotherapy is applied in combination with AAV-2 infection. *In vitro* proliferation assays indicated that the number of proliferating cells, after combined treatment with cisplatin and etoposide, can be significantly reduced by concomitant AAV-2 infection, as compared with treated but non-infected controls. H446 SCLC cells, which show resistance to etoposide/cisplatin chemotherapy (compared with a cell line which was never chemotherapeutically treated before, like NCI-H209) were significantly more sensitive after AAV-2 infection, suggesting that the therapeutic efficacy of chemotherapy in SCLC can be enhanced even if the cells are already relatively resistant to chemotherapy. Similarly, *in vivo* growth of tumours induced by inoculation of SCLC cells into immunocompromised nude mice was reduced more efficiently in AAV-2-infected animals compared with tumours in mice treated with chemotherapeutic drugs alone. These data extend and further support our previous reports on AAV functions which might be useful in improving the efficacy of chemotherapeutic drugs used in human cancer treatment.

Key words: AAV, chemotherapy, human tumour cells, parvovirus, small cell lung cancer cells
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INTRODUCTION

ADENO-ASSOCIATED VIRUS type 2 (AAV-2) is a human parvovirus that requires co-infection with a helper virus for productive infection to take place. AAV-2 infects humans early in childhood and is believed to be apathogenic since no human disease has been associated with AAV-2 infection [1]. On the contrary, as with other members of the parvovirus family, AAV-2 actually exhibits tumour suppressive properties (reviewed in [2]). In addition, we have shown that AAV-2 sensitises human tumour cell lines and freshly established human tumour cells to gamma irradiation and to chemotherapeutic drugs, *in vitro* and *in vivo* [3, 4].

Small cell lung cancer (SCLC) is highly sensitive to the first cycle of chemotherapy. However, in almost all patients chemotherapy-resistant SCLC cells are selected, which resist further chemo- or radiotherapy leading to rapid death of the respective patients [5, 6]. Selection of resistant tumour cells is determined by the number of tumour cells which are not killed by primary treatment but survive under conditions of further genotoxic damage. We were, therefore, interested to test whether infection with AAV-2 reduces the selection of

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chemotherapy resistant SCLC cells by enhancing the cytotoxic effect of chemotherapeutic drugs in cell culture and in tumours established in immunocompromised mice. Our results presented here indicate that infection with AAV-2 significantly increases the efficacy of chemotherapeutic treatment in SCLC tumour cells and tumours.

MATERIALS AND METHODS

Cells

SCLC cell lines (NCI-H69, NCI-H146, NCI-H209 and NCI-H446) [7, 8] were grown in RPMI 1640 medium (Eurobio, Raunheim, Germany). HeLa cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) (Eurobio). All growth media were supplemented with glutamine (Eurobio; 1%), antibiotics (penicillin and streptomycin) and 10% heat-inactivated fetal calf serum (PAA, Linz, Austria). The cultures were incubated at 37°C in a humidified atmosphere with 5% CO_2, and routinely tested for mycoplasma contamination.

Virus

AAV-2 was propagated in HeLa cells using adenovirus type 2 (Ad-2) as a helper. AAV-2 was purified on a caesium chloride gradient and titrated as described previously [9]. Purified AAV-2 was checked for residual infectious Ad-2 as described previously [10]. Ad-2 inocula were cleared supernatants of Ad-2-infected HeLa cells.

Infection protocol

SCLC cells were suspended in phosphate-buffered saline (PBS) and incubated with purified AAV-2 at the multiplicities of infection (MOI) indicated. After 45 min (at 37°C), non-absorbed virus was removed by washing with PBS and growth medium was replenished. As mock-infection controls, we used either PBS or the heat-inactivated fraction (56°C, 30 min) of a caesium chloride gradient of cells infected with Ad-2 only, with the density (1.14 g/cm^3) of the AAV-2 containing fraction used in the AAV-2 purification, as indicated in the respective experiments. The volume/cell ratio of the latter was 50-fold higher (5 ml/10^6 cells) than that used for AAV-2 infections.

Treatment with chemotherapeutics

AAV-2- or mock-infected cells were treated with cisplatin (Asta Medica, Frankfurt/Main, Germany) or etoposide (Bristol, München, Germany) or both (ratio cisplatin:etoposide 1:2.5) by adding drugs dissolved in PBS to the medium at the concentrations indicated.

In vitro *proliferation assay*

Proliferation of SCLC cells after infection with AAV-2 and/or treatment with chemotherapeutics was determined by a modified MTT (3-(4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazoliumbromide) assay [11]. After infection or mock-infection, cells were seeded in 24 well plates at a density of 10^5 cells/well and treated with chemotherapeutics at the concentrations indicated. After 6 days (NCI-H69, NCI-H446) or 8 days (NCI-H146, NCI-H209) MTT (Sigma, Deisenhofen, Germany) was added to the culture medium to a final concentration of 0.5 mg/ml. The cultures were then incubated for 4 h at 37°C to allow the reduction of MTT to blue formazan (mediated by mitochondrial dehydrogenases) [12] indicating active proliferation of the cells. The cells were centrifuged, washed with PBS and the formazan solubilised with isopropanol. Precipitated proteins were pelleted by centrifugation (1000 rpm; 15 min) and 200 µl samples of the supernatant were measured to determine the optical density at 540 nm (OD_{540}) using OD_{690} as reference, with a Titertek Multiskan plus MKII (Lab Systems, Finland) densitometer.

Relative proliferation (A/A_0) was defined as the ratio of absorption measured in the supernatant of AAV-2-infected and/or drug-treated cells (A) compared with the absorption measured in the supernatant of mock-infected and untreated control cells (A_0). The IC_{50} value was defined by drug concentrations leading to a 50% inhibition of proliferation.

Cells infected with AAV-2 (MOIs indicated) or mock-infected were seeded into 24-well plates, and treated with cisplatin. After 6 or 8 days, respectively, relative proliferation was determined. This protocol was based on experiments to define conditions of exponential cell growth for each cell line. In addition, kinetic studies were performed to determine optimal treatment modalities for SCLC cells after AAV-2 infection. In these studies we have shown that AAV-2-mediated sensitisation was maximal 1–3 h post infection [10]. In the present study, chemotherapeutics were added 3 h after AAV-2-infection, since this time point had also been chosen in previous studies [4]. To exclude effects mediated by factors still present after caesium chloride gradient purification and Ad-2 heat inactivation, a mock-infection control with PBS was used in addition to the mock-infection with the respective gradient fraction of a cell lysate of cells infected with Ad-2.

Southern blot analysis

Total DNA was prepared from infected and mock-infected cells as described previously [4] at the indicated time points.

Animal experiments

Female nude mice (CD1-nu/nu) purchased from Iffa Credo (Brussels, Belgium) were kept in isolators and received food and water *ad libitum*. Exponentially growing SCLC cells (NCI-H209) were injected subcutaneously into the flanks of 6-week old mice (10^7 cells in 100 µl of PBS per animal). Five months after the inoculation of the cells, when the tumours had reached an average volume of 200 mm^3, the animals were infected weekly with AAV-2 (intratumoral injection of 10^8 tissue culture infectious doses [TCID]) and/or treated with chemotherapeutics by intraperitoneal injection (3 mg/kg body weight cisplatin [weekly] and 7.5 mg/kg body weight etoposide [three times a week]). Details of the onset and cessation of treatment are indicated in Figure 1. In each group (control, chemotherapeutic treatment, AAV-2 infection, treatment + infection), five animals were included. Infected and non-infected animals were kept in separate isolators. Tumour diameters were measured weekly and the tumour volume was determined by the formula: tumour volume = 1/2×width×depth×height [13]. The relative tumour volume (V/V_0) was determined for each animal and time point (ratio of the tumour volume [V] compared with the tumour volume at the beginning of treatment [V_0]).

RESULTS

Sensitivity of SCLC cell lines to cisplatin and etoposide

We performed MTT *in vitro* proliferation assays with various concentrations of cisplatin and/or etoposide as described in Materials and Methods to determine the sensitivity of SCLC cell lines to cisplatin and/or etoposide. Combined treatment with both drugs was performed according to

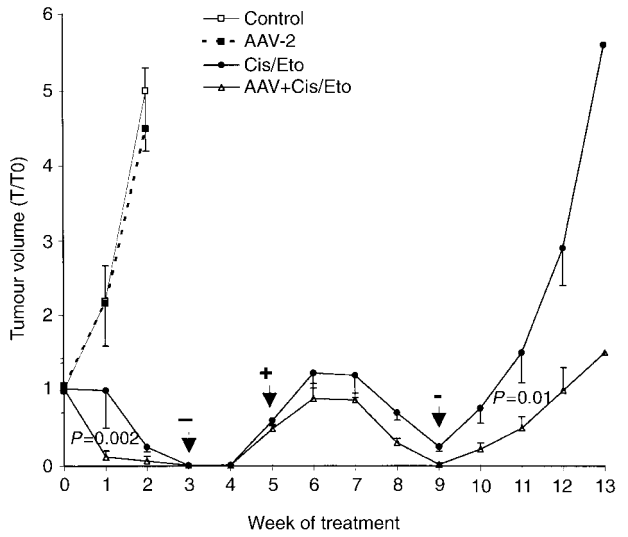

Figure 1. Adeno-associated virus (AAV)-mediated sensitisation of NCI-H209 cell-derived tumours established in nude mice (means of five animals per group). The cisplatin dose was 3 mg/kg body weight (administered weekly), the etoposide dose was 7.5 mg/kg body weight (administered three times a week); 10^8 TCID (tissue culture infectious dose)/animal (weekly) of infectious AAV-2 particles were applied. Arrowheads indicate change in treatment modalities: –, interruption of drug treatment and AAV-2 infection; +, onset of treatment and infection. *P* values were determined by Student's *t*-test. Error bars indicate the standard deviation.

commonly used clinical protocols [5]. As shown in Table 1, the SCLC cell lines NCI-H69 and NCI-H446 exhibited high intrinsic resistance to both drugs, with low levels of sensitivity to cisplatin/etoposide treatment, whereas NCI-H146 cells were highly sensitive for etoposide and NCI-H209 cells were highly sensitive for both drugs.

MOI dependence of AAV-2-mediated drug sensitisation of SCLC cell lines

In order to determine whether infection with AAV-2 affects the proliferation rate of SCLC cells and/or sensitises these tumour cells to the cytotoxic action of chemotherapeutics, we measured the relative proliferation of NCI-H209 and NCI-H446 SCLC cells after infection with different MOIs of AAV-2 (10^1–10^5 TCID/cell) with or without subsequent treatment with cisplatin at IC_{50} as listed in Table 1. As shown in Figure 2(a) and (b), AAV-2 infection led to a decrease in the proliferation rate of cisplatin-treated cells at a MOI of 10^3 or 10^4 TCID/cell. Infection with a MOI of 10^5 TCID/cell did not result in a further enhancement of the effect. No significant inhibition of proliferation was observed after infection with lower MOIs of AAV-2 or after mock-infections, indicating a specific effect due to infection with high MOI of AAV-2. The relative proliferation of AAV-2-infected ($10^{3–5}$ AAV-2/cell) and cisplatin-treated (IC_{50}) cells was decreased to 0.25 in NCI-H446 and 0.29 in NCI-H209 cells compared with a relative proliferation in only cisplatin (IC_{50})-treated cells (0.51 in NCI-H446 and 0.59 in NCI-H209 cells).

Table 1. Concentration of chemotherapeutics resulting in a 50% inhibition of proliferation (IC_{50}) of small cell lung cancer cell lines

Cell line	Cisplatin IC_{50} (μg/ml)	Etoposide IC_{50} (μl/ml)	Cisplatin/etoposide IC_{50} (μg/ml)
NCI-H69	0.2	0.26	0.08/0.2
NCI-H146	0.11	0.025	0.008/0.02
NCI-H209	0.007	0.053	0.006/0.015
NCI-H446	0.15	0.21	0.042/0.105

The drug concentration of cisplatin, etoposide or both leading to 50% inhibition of proliferation (IC_{50}) was determined using the *in vitro* proliferation assay (MTT).

Quantification of AAV-2-mediated drug sensitisation of SCLC cell lines

To quantitate the sensitisation of cells to chemotherapeutics after infection with AAV-2, dose–response curves were established. We determined the relative proliferation of the cell lines after mock-infection (PBS) or AAV-2 infection with 10^3 or 10^4 TCID/cell and subsequent treatment with various concentrations of cisplatin or etoposide or a combination of both drugs (Table 2). Sensitisation factors (SF) were defined as the ratio of the IC_{50} values of infected cells compared with the IC_{50} values of mock-infected cells. The SF indicates the factor by which a concentration of a chemotherapeutic agent can be lowered after infection with AAV-2 to achieve the same extent of inhibition of proliferation. As summarised in Table 2, sensitisation by AAV-2 was moderate in NCI-H69 and NCI-H146 (maximal SF around 1.4 at a MOI of 10^4 TCID/cell). Infection of NCI-H209 or NCI-H446 induced a more significant sensitisation in a MOI-dependent manner (maximal SF of around 3 (NCI-H446) and 2.3 (NCI-H209) at a MOI of 10^4 TCID/cell). *P* values determined by Student's *t* test were highly significant for both cell lines (see Table 2). AAV-2-mediated sensitisation was not dependent on the chemotherapeutic drug used.

Drug-induced helper virus-independent AAV-2 DNA replication

As shown in previous studies, exposure to genotoxic stress can induce helper virus-independent replication of viral DNA in AAV-infected cells [14–17]. We analysed all four SCLC cell lines for drug-induced AAV DNA replication by Southern blot analysis. Drug treatment-mediated helper virus-independent amplification of AAV-2 DNA could only be

Table 2. Sensitisation factor of small cell lung cancer cell lines treated with cisplatin and/or etoposide and infected with adeno-associated virus

Cell line	Chemotherapeutic drug (IC_{50})	Sensitisation factor (A/A_0) 10^4 TCID/cell
NCI-H69	Cisplatin	1.43
	Etoposide	1.30
	Cisplatin/etoposide	1.45 ($P = 0.01$)
NCI-H146	Cisplatin	1.37
	Etoposide	1.38
	Cisplatin/etoposide	1.33 ($P = 0.01$)
NCI-H209	Cisplatin	2.33
	Etoposide	2.12
	Cisplatin/etoposide	2.00 ($P = 0.0002$)
NCI-H446	Cisplatin	2.50
	Etoposide	3.00
	Cisplatin/etoposide	3.00 ($P = 0.0001$)

*Relative proliferation (A/A_0) was calculated by the ratio of the absorbance of adeno-associated virus type 2 (AAV-2)-infected and/or drug-treated (A) to the absorbance of mock-infected, untreated controls (A_0). *P* values show the statistical significance of etoposide/cisplatin-treated cells compared with etoposide/cisplatin-treated and AAV-infected cells. TCID, tissue culture infectious dose.

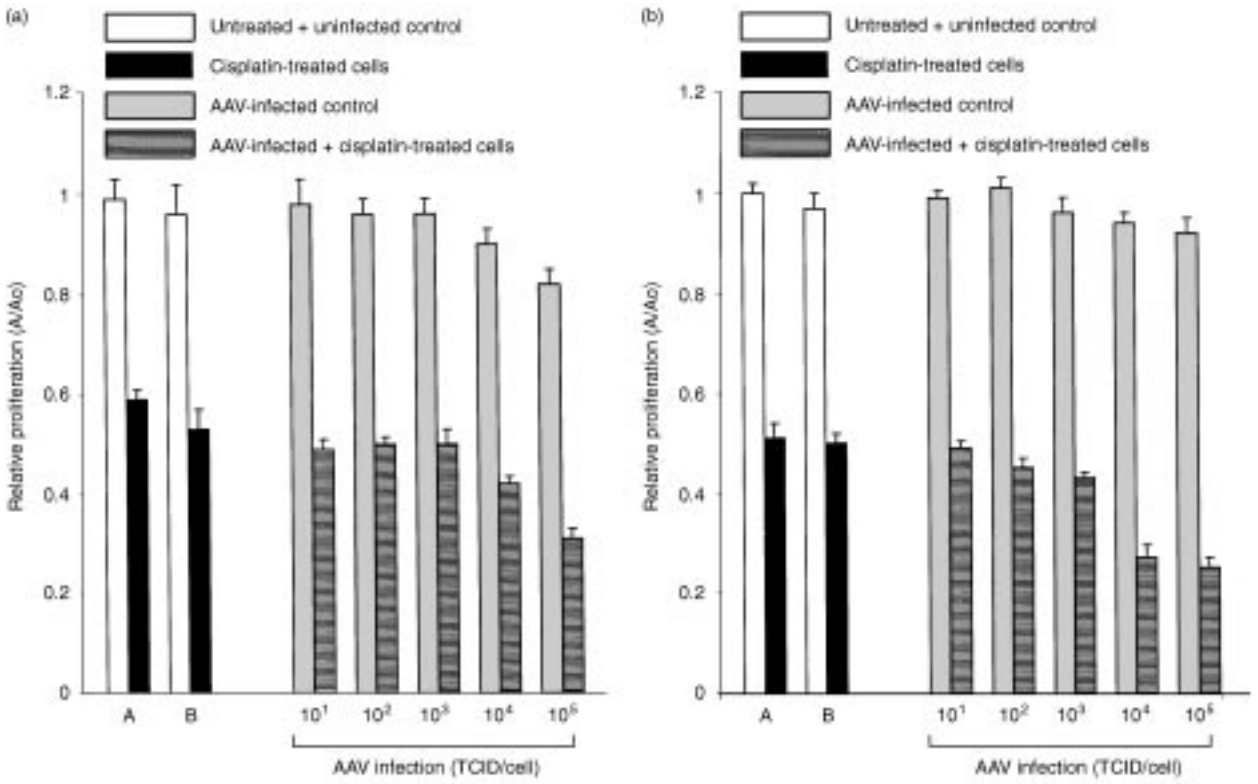

Figure 2. Multiplicities of infection (MOI) dependence of adeno-associated virus (AAV)-mediated sensitisation of small cell lung cancer (SCLC) cell lines to cisplatin. Relative proliferation (A/A_0) of the SCLC cell lines, NCI-H209 (a) and NCI-H446 (b) after mock-infection (A, phosphate buffered saline alone; B, infected with heat-inactivated gradient of Ad-2-infected cells, see Materials and Methods) or infection with different MOI of AAV-2 with or without subsequent treatment with IC_{50} of cisplatin as listed in Table 1. TCID, tissue culture infectious doses. Mean values of six independent experiments. Error bars indicate the standard deviation.

detected in NCI-H446 cells, whereas no AAV-2 DNA replication was detectable in all other cell lines (data not shown).

AAV-2-mediated drug sensitisation of NCI-H209-derived tumours in nude mice

In order to analyse whether AAV-2-mediated sensitisation of SCLC cell lines can also be achieved *in vivo*, we inoculated NCI-H209 cells (and in another animal experiment NCI-H446 cells) into the flanks of immunocompromised mice. (Since the tumour growth of inoculated NCI-H446 cells was extremely low, we were not able to produce enough tumour bearing animals to evaluate the experiment.)

Tumour bearing animals were infected with AAV-2 (10^8 TCID/animal) and/or were treated with a combination of cisplatin and etoposide (3 mg/kg body weight cisplatin, weekly, and 7.5 mg/kg body weight etoposide, three times a week) at the times indicated in Figure 1. Previous studies had shown that 10^8 TCID of AAV particles per animal are sufficient to induce AAV-mediated sensitisation in mice [4]. As shown in Figure 1, treatment with chemotherapeutics resulted in a rapid decrease in tumour volumes and complete regression after 3 weeks of treatment. The combination of chemotherapy with AAV-2 infection led to a more pronounced decline of tumour volumes compared with animals that received only chemotherapy, indicating a sensitisation of drug-treated tumour cells by AAV-2 ($P = 0.002$; after 1 week of treatment). Infection with AAV-2 alone had no significant effect and tumour volumes increased at the same rate as untreated controls. Treatment was interrupted after complete regression of the tumours and was resumed when relapses occurred. Treatment of recurrences was less efficient in animals that received solely drug treatment compared with those occurring in AAV-infected and chemotherapeutically-treated animals ($P = 0.01$; week 11). This indicates that development of resistance to the initial treatment had occurred, at least in the chemotherapeutically treated animal group. Recurrent tumours in AAV-2 infected animals continued to be sensitive to treatment with cisplatin and etoposide, but tumour regression was slower compared with the regression of the initial tumours. At week 9, in three out of five animals infected with AAV-2, tumours regressed completely, in contrast to the tumours in animals treated with chemotherapeutics alone in which complete regression of tumours was not observed. Furthermore, AAV-2-infected and cisplatin/etoposide-treated animals were in a better physical condition (e.g. no cachexia) than only cisplatin/etoposide-treated animals. Macroscopic pathological examination did not reveal any side-effects associated with AAV-2 infection.

DISCUSSION

The treatment of SCLC in humans is characterised by initially reasonable responses to chemotherapy followed by rapid development of drug resistance and finally leading to a high mortality in patients suffering from SCLC [5, 18, 19]. Therefore, new approaches need to be developed to improve the efficiency of such treatments.

To assess whether the previously reported AAV-mediated sensitisation of tumour cells to chemotherapy [4] might be

envisaged to overcome the therapeutic problems in the treatment of lung cancer, we tested cells of four different human SCLC cell lines *in vitro* and *in vivo*. In these cell lines, significantly increased sensitivity to cisplatin and etoposide could be observed *in vitro* and improvement of therapeutic efficacy was achieved *in vivo*.

NCI-H209 is a cell line derived from a tumour that was not treated by chemotherapeutic agents prior to cultivation and is not resistant to the drugs. NCI-H209 cells are, as described in Materials and Methods, SCLC cells that originate from a patient who had not received chemotherapy. Therefore, animal experiments performed with this cell line reflect the clinical situation of SCLC patients undergoing chemotherapy. In contrast, NCI-H446 was established from a resistant cancer, but also proved to become re-sensitised to cisplatin/etoposide following AAV infection. In view of the fact that AAV infection can prevent gene amplification-mediated resistance in model systems *in vitro* [14, 20], it is tempting to speculate that AAV infection may also interfere with the induction of resistance *in vivo*.

The mechanisms of AAV-mediated sensitisation and enhanced killing of tumour cells remain to be determined. We could not detect any AAV-specific effect on the expression of the cellular genes c-*myc*, *p53*, *Rb* and *NSE* in the drug-treated cells of the SCLC lines tested [10]. In addition, viral gene expression in drug-treated and infected SCLC cells could not be detected (except in NCI-H446 cells that exhibit AAV DNA replication upon treatment with cisplatin/etoposide), suggesting that novel synthesis of viral macromolecules is not essentially required for sensitisation. This suggests that interaction of viral particles with the cellular membrane may trigger specific cellular responses or that DNA molecules of the infectious virion interact with cellular factors leading to modulation of the cell's growth rate. It is also conceivable that AAV infection in combination with cytotoxic stress might induce synthesis of cytokine-like molecules triggering sensitisation in neighbouring cells. Recent data indicate that infection with AAV of drug-treated human tumour cells leads to the enhancement of drug-mediated apoptosis [21]. Further experiments are required to unravel the molecular mechanism of AAV-mediated sensitisation.

More experiments are needed to determine, for example, the optimal amount of virus, the most effective route of delivery and the disease types that could be treated. However, even before fully understanding the underlying mechanisms associated with this phenomenon, it might be justified to envisage clinical trials on tumours showing a poor response to chemotherapy, since pathogenic effects are not to be expected from AAV infection and since expression of viral gene products appears not to be necessary.

1. Berns KI, Bohensky RA. Adeno-associated viruses: an update. *Adv Virus Res* 1987, **32**, 243–306.
2. Schlehofer JR. The tumour suppressive properties of adeno-associated viruses. *Mutat Res* 1994, **305**, 303–313.
3. Walz C, Schlehofer JR, Flentje M, Rudat V, zur Hausen H. Adeno-associated virus sensitizes HeLa cell tumours to gamma-rays. *J Virol* 1992, **66**, 5651–5657.
4. Klein-Bauernschmitt P, Knebel Doeberitz M von, Ehrbar M, Geletneky K, Kleinschmidt J, Schlehofer JR. Improved efficacy of chemotherapy by parvovirus-mediated sensitization of human tumour cells. *Eur J Cancer* 1996, **32A**, 1774–1780.
5. Ihde DC, Pass HI, Glatstein EJ. Small cell lung cancer. In de Vita V, Hellman S, Rosenberg SA, eds. *Cancer Principles and Practice of Oncology*. Philadelphia, Lippincott, 1993, 723–758.
6. Blackstein ME. Advances in chemotherapy for small cell lung cancer. *Semin Oncol* 1994, **21**, 38–42.
7. Gazdar AF, Carney DN, Russel EK, *et al.* Establishment of continuous, clonable cultures of small cell carcinoma of the lung which have amine precursor uptake and decarboxylation cell properties. *Cancer Res* 1980, **40**, 3502–3507.
8. Carney DN, Gazdar AF, Bepler G, *et al.* Establishment and identification of small cell lung cancer cell lines having classic and variant features. *Cancer Res* 1985, **45**, 2913–2923.
9. Botquin V, Cid-Arregui A, Schlehofer JR. Adeno-associated virus type 2 interferes with early development of mouse embryos. *J Gen Virol* 1994, **75**, 2655–2662.
10. Hillgenberg M. Einfluβ der Infektion mit Adeno-assoziierten Viren auf die Chemotherapeutika-Sensitivität kleinzelliger Bronchialkarzinomzellen *in vitro* und *in vivo*, PhD Thesis, Rheinische Friedrich-Wilhelms-Universität Bonn, 1995.
11. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983, **56**, 55–63.
12. Berridge MV, Tan AS. Characterization of the cellular reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): subcellular localization, substrate dependence, and involvement of mitochondrial electron transport in MTT reduction. *Arch Biochem Biophys* 1993, **303**, 474–482.
13. Tomayko MM, Reynolds CP. Determination of subcutaneous tumour size in athymic (nude) mice. *Cancer Chemother Pharmacol* 1989, **24**, 148.
14. Schlehofer JR, Heilbronn R, Georg-Fries B, zur Hausen H. Inhibition of initiator-induced SV40 gene amplification in SV40-transformed Chinese hamster cells by infection with a defective parvovirus. *Int J Cancer* 1983, **32**, 591–595.
15. Schlehofer JR, Ehrbar M, zur Hausen H. Vaccinia virus, herpes simplex virus, and carcinogens induce DNA amplification in a human cell line and support replication of a helpervirus dependent parvovirus. *Virology* 1986, **152**, 110–117.
16. Yakobson B, Koch T, Winocour E. Replication of adeno-associated virus in synchronized cells without the addition of a helper virus. *J Virol* 1987, **61**, 972–981.
17. Yalkinoglu AO, Heilbronn R, Bürkle A, Schlehofer JR, zur Hausen H. DNA amplification of adeno-associated virus as a response to cellular genotoxic stress. *Cancer Res* 1988, **48**, 3123–3129.
18. Minna JD, Pass H, Glatstein E, Ihde DC. Lung Cancer. In DeVita VT, Hellmann S, Rosenberg SA, eds. *Cancer Principles and Practice of Oncology*. Philadelphia, Lippincott, 1989, 591–705.
19. Cook RM, Miller YE, Brunn PA. Small cell lung cancer: etiology, biology, clinical features, staging, and treatment. *Cur Probl Cancer* 1993, **17**, 71–141.
20. Yalkinoglu AO, Schlehofer JR, zur Hausen H. Inhibition of N-methyl-N′-nitro-N′-nitrosoguanidine-induced methotrexate and adriamycin resistance in CHO cells by adeno-associated virus type 2. *Int J Cancer* 1990, **45**, 1195–1203.
21. Klein-Bauernschmitt P, Schlehofer JR, von Knebel Doeberitz M. Infection with adeno-associated virus type 2 enhances apoptosis of cisplatin-treated human tumour cells. *Virus Res* 1997, **47**, 133.

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